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BY

F. T. BROOKS, M.A.

(Senior Demonstrator of Botany, Cambridge University, and formerly Government Mycologist, Federated Malay States.)

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Introduction.

Soon after arriving in the Federated Malay States at the beginning of 1914, the writer frequently saw a disease of rubber trees which had not hitherto been recorded in Malaya, the disease affecting primarily the collar and root system of the trees. Preliminary investigation shewed that the trouble was probably caused by a fungus, as hyphae were abundant along the junction of diseased and healthy tissues. The characters of this disease were different from those of the root diseases caused by *Fomes semitostus* (now more correctly named *Fomes lignosus*), *Sphærostilbe repens* and *Hymenochæte noxia*, but it was not until towards the end of the year that I found the fructifications of the causative fungus, which proved to be *Ustulina zonata* (Lev.) Sacc. This fungus is the cause of a common root disease of tea in Ceylon (4), and although Petch (5) wrote in 1911 that "it is not yet certain that *Ustulina* causes root disease in *Hevea*," he pointed out in 1914 (6) that several cases of *Ustulina* on rubber had been reported in Ceylon in fields where *Hevea* had been planted among tea which had subsequently been allowed to die out. This is the only reference to the occurrence of *Ustulina zonata* on rubber I have been able to find in published accounts of the diseases of *Hevea brasiliensis*, and no general account of the manner in which it attacks this host has hitherto appeared. The disease has not yet been recorded on *Hevea* in Java, Sumatra or Borneo.

Field Observations.

The part of the rubber tree chiefly affected by this disease is the collar, which is usually attacked first on one side only. In this region the bark dies and the wood below becomes brown. As the dead tissues break away a shallow depression is often formed on one side of the trunk at soil level. Neighbouring lateral roots and the portion of the tap root immediately below the collar are often affected in the same manner, and in advanced

cases the disease spreads up the trunk to the height of two or three feet. If the diseased tissues are exposed, conspicuous black lines (cf. Fig. 3) about $\frac{1}{32}$ inch in diameter are often seen near the limits of the affected parts, though these lines are not invariably present. These narrow black zones occur more frequently in the wood than in the bark and their course is rather irregular. No external mycelium has been found associated with roots suffering from this disease, hence it is readily distinguished from the root troubles caused by *Fomes semitostus* (*Fomes lignosus*) and by *Hymenochæte noxia*. The absence of rhizomorphic strands between the bark and the wood distinguishes the affection from that caused by *Sphærostilbe repens*, the black lines in the tissues often associated with *Ustulina zonata* being chiefly found in the wood and differing in nature from rhizomorphic strands, as will be shewn below.

This disease, which is by no means uncommon in Malaya, principally affects old trees, and some of the finest specimens of plantation rubber 14 to 16 years of age have succumbed to it. A few trees five to six years old have also been attacked by it. The disease has been found both on flat and on undulating land in several parts of the country. It is, however, only in the few estates where groups of trees have been killed by it that serious damage has yet been done by this fungus.

As the fungus progresses in the collar and root system of the tree, the foliage becomes thin and the branches commence to die back; the tree gradually dies unless successfully treated in an early stage of attack.

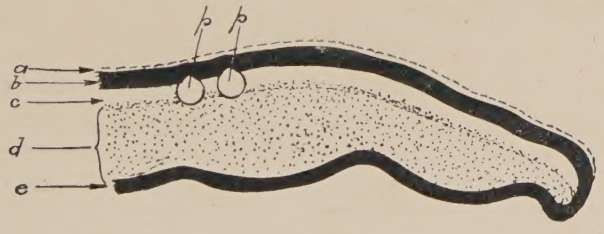
Fructifications of the fungus appear on dead tissues of the collar and exposed roots of affected trees to which they are usually closely adpressed. The fruit-bodies which were found on several occasions on diseased rubber trees in various parts of the country towards the end of 1914, are plate-like in form and grey brown to blackish in colour. The fructifications are easily overlooked, especially in the earlier stages of development, for the colour is not unlike that of the soil on many estates. The difficulty of distinguishing the fungus is further increased in wet weather, because the fructifications developing near the ground become splashed with mud. Fig. 1 shews a fruit body which was taken from a diseased rubber tree. Fig. 2 is a photograph of the base of a diseased rubber tree bearing a fructification (*f*) of *Ustulina zonata* at about soil level. The tree has been opened up in order to shew the limits of diseased and healthy tissues (*d* and *h* respectively) above the collar.

Description of the Fungus.

In Malaya the fruit bodies of the fungus arise as small greyish brown plates on the exposed lateral roots or collar of the affected tree, the growing margin of the fructifications being

usually greenish. Petch (4) describes the occurrence of a conidial stage when the fruit body is young, in which condition the surface is covered with a white felt consisting of slender, closely packed conidiophores, which abstrict small, hyaline spores from their extremities. I have not found this conidial stage on young fructifications kept under observation in Malaya, though of course it may be subsequently found. Mr. T. Petch has, however, kindly sent me some specimens in the conidial condition taken from a Mango tree in Ceylon, from which Fig. 4 has been drawn. The conidia are about $4\mu \times 2\mu$ in size. These fructifications in the conidial stage from Ceylon are much lighter in colour than the fructifications devoid of conidia of about the same age obtained in the Federated Malay States. Adult fruit bodies gathered in Malaya have been compared with mature specimens of *Ustulina zonata* from Ceylon and other countries in the herbaria at Kew and at Peradeniya with which they are undoubtedly identical, hence there is no hesitation in naming the fungus *Ustulina zonata* (Lev.), Sacc.

The plate-like fructifications of *Ustulina zonata* are several inches across when fully developed and are marked by an irregular and obscurely zoned surface, which is punctured by minute black dots scarcely visible to the naked eye. The outline of the fruit body is irregular and the centre is often roughly corrugated. Petch (4) points out that the fungus is extremely variable, and says that the fructifications are sometimes stalked. The variation in the Malayan specimens is also a marked feature. If the grey brown surface of the fructification is scratched, a black layer is seen which is also exposed at times by the natural wearing away of the thin covering. The fruit bodies though rather soft when young are brittle when mature; they are about $\frac{1}{8}$ inch thick, or rather less. The accompanying diagram, which is about eight times natural size, illustrates the different layers seen in section when a mature fructification is broken across.



On the upper surface (*a*) represents the remains of the greyish brown film which, in the Malayan specimens so far examined, is sterile, but in the Ceylon specimens gives rise to the conidial

stage. (*b*) is the upper black zone which sometimes becomes exposed by the wearing away of the greyish brown film above. (*c*) is a white layer in which the globular perithecia (*p*) are formed. (*d*) is a broader band grey in colour and leathery in consistency. (*e*) is the black zone on the under surface, which is continuous at the margin with the black zone towards the upper surface. The black zones are brittle. The perithecia (*p*) are globose and communicate with the exterior by exceedingly narrow channels. The minute black dots seen on the fructification after the superficial grey brown layer has been worn away correspond to the ostioles of the perithecia. They are raised very slightly above the general level of the surface of the fruit body and are formed before the perithecia are fully organised. Each ascus contains eight black spindle-shaped spores which are somewhat inequilateral (cf. Fig. 5). The dimensions of these spores are 28–32μ × 7–10μ. There are large numbers of thread-like paraphyses between the asci. The ripe spores exude from the perithecia under moist conditions. When a portion of a fructification is kept damp in a Petri dish the spores are liberated and look like drops of ink spread over the surface. The ascus walls deliquesce before the extrusion of the spores from the perithecia as in other members of the *Xylariaceæ* to which the genus *Ustulina* belongs; this behaviour of the ascus walls is probably an important factor in bringing about the liberation of the spores.

The genus *Ustulina* is distinguished from other members of the *Xylariaceæ* which have flat fructifications, by the fact that the fruit bodies are rather soft when young and are covered by an evanescent layer which often forms conidia before the elaboration of the perithecia below. In the closely allied fungus *Daldinia concentrica*, the superficial layer of the young fructifications does not always form conidia, so it is not remarkable to find that the formation of conidia by the corresponding layer in *Ustulina zonata* is not constant.

The species *Ustulina zonata* was first described under the name of *Sphæria zonata* by Léveillé (3) in 1845 from material obtained on palms in Java. It has since been placed in the genus *Ustulina* by Saccardo (7).

Effect of the Fungus on the Tissues of Hevea.

Wood and bark invaded by the fungus become discoloured, and near the margin of the affected tissues conspicuous black lines are often present, these being caused by the dense aggregation and darkening of the hyphae in the cells of these regions. Smaller black zones sometimes occur as islands a little distance away from the longer lines. Fig. 3 shews a general view of one of these black lines in the wood, which are closely similar to the black zones in the fructifications. Fig. 6 shews a section through one of these black lines seen under the microscope. The

brown tissues bordering these black lines contain hyaline hyphae, which are especially abundant in the vessels. It is noteworthy that the black lines often occur just within the margin of the region penetrated by the hyphae. On one occasion tyloses were found in vessels affected by the fungus, but the presence of these in-growths does not occur regularly as in the tissues of *Hevea* invaded by *Corticium salmonicolor*, as described by Brooks and Sharples (1). These conspicuous black zones are caused by the fungus forming a kind of sclerotic plate in the tissues, the difference between this aggregation and a typical sclerotium being that the latter does not include within it portions of the tissues of the host. These black zones differ from rhizomorphic strands in the same respect. The fungus invades the tree either in the collar or in one of the roots, from either of which it advances, spreading more rapidly in a longitudinal than in a lateral direction. In advanced stages of disease several of the laterals and the tap root may be dead and the fungus may have progressed two or three feet up the trunk of the tree. In the latter case boring beetles often attack the affected parts of the trunk, as also happens sometimes with trees affected by *Sphærostilbe repens*. The presence of black lines in the roots of tea attacked by *Ustulina zonata* is mentioned by Delacroix and Maublanc (2).

The presence of black lines in dead rubber wood must not, however, be considered a certain sign that *Ustulina zonata* is present, as several of the fungi belonging to the *Xylariaceæ* are of common occurrence as saprophytes on dead branches and roots of rubber trees, and two of these, *Nummularia pithodes* (B. and Br.), Petch, and a species of *Xylaria*, have been found by the writer to produce similar black lines in the wood. On one occasion this species of *Xylaria* was seen to affect living tissues of exposed *Hevea* roots which had been badly wounded some time previously, possibly by changkols or through being gnawed by animals. This fungus had entered the wounds, and after setting up decay had advanced into the living tissues to some extent, although no serious damage was being done. It is only when these black lines in the wood and bark are associated with an extensive affection of the collar and root system of the tree that *Ustulina zonata* should be suspected.

Pure Cultures of the Fungus.

Pure cultures were established from the mycelium present in one of the black zones taken from the collar of an affected rubber tree. In order to do this, small portions of the black plate were cut out under sterile conditions and placed on blocks of *Hevea* wood in culture tubes. In a short time a greyish mycelium began to grow from the black mass and spread slowly over the block of wood. This mycelium was fine in texture, and remained closely adpressed to the wood. At a later stage the mycelium in contact with the wood became black, and, together

with the mycelium in the superficial layers of the wood, formed a continuous black crust similar to the black zones in the collar of a diseased tree. In some of the cultures a black line was formed in the wood block a little within the surface. Fig. 7 shews a section of one of these black zones in a block of wood on which the fungus grew in pure culture.

Since returning to England I have found that the fungus grows well on sterilised blocks of ash wood kept at a temperature of 25° C. At 14° C., however, it grows scarcely at all.

Inoculation Experiments.

After pure cultures of the fungus had been obtained, inoculation experiments were carried out upon seedling rubber plants growing in pots and upon trees about four years of age situated on the slope of a hill. Blocks of wood on which the fungus was growing in pure culture were tied or placed against the main root of the seedling a little below the level of the soil. With the four year old trees either the main root or one of the larger laterals was inoculated in the same way. In three of the seedling plants thus inoculated the wood block bearing the fungus was placed against a wound made in the root with a scalpel; in three others the fungus was tied against the uninjured surface. A month later the leaves of one of the inoculated and unwounded plants began to droop, and, shortly after, investigation shewed that the plant was diseased at the collar, and there was clear indication that the fungus had spread from the wood block into the plant. Sections shewed a brown discolouration of the wood and bark of the root and collar above the place against which the wood block had been tied, but no black lines were present in the tissues. The pith was rotten for two inches upwards from the region of inoculation, and a hyaline mycelium was densely aggregated along the line of the cambium, where it formed a kind of pseudoparenchyma.

A week later, *i.e.*, five weeks after inoculation, two other seedling plants, both of which had been wounded, began to droop, and examination shewed that they were affected in the same way as the seedling described above. The three other plants remained healthy. The third wounded plant, which did not become affected, shewed only a slight discolouration around the wound, which was already partly healed over. Thus in this series of experiments the fungus entered and successfully infected one unwounded plant and two wounded ones.

Of the fourteen four year old trees which were inoculated, eight were wounded before inoculation and six were not injured. The wounds made in these trees reached as far as the wood. Six weeks after inoculation the trees were examined, when it was found that the fungus had made considerable progress in each one of the wounded roots that had been inoculated, but, with one doubtful exception, it had not penetrated the roots

which had not been injured. The wood around the wounds, through which the fungus had entered, was markedly discoloured. Control wounds against which the fungus was not placed shewed much less discolouration in the same period, hence the effect upon the inoculated tissues was not due to the entrance of other micro-organisms from the soil. The absence of infection in the uninjured roots of these four year old trees is certainly striking, although, as described above, the fungus succeeded in invading the uninjured surface of the root of one of the seedling plants.

Treatment.

It has already been pointed out that in Malaya this disease chiefly affects old rubber trees. It is likely that the death of old plantation trees has often been attributed to *Fomes semitostus* when the loss was really caused by *Ustulina zonata* or by *Sphærostilbe repens*, which also attacks mature trees more frequently than young ones. *Fomes semitostus* does not often attack old rubber trees, partly because by the time a plantation is ten years old this fungus has generally worked itself out if the estate has received proper treatment. *Fomes* is the chief foe of young rubber because it begins to develop on jungle stumps soon after they begin to decay, whence it spreads to the roots of young rubber plants.

Petch states that *Ustulina zonata* is the commonest root disease of tea in Ceylon, and says it is specially prevalent in plantations in which *Grevillea* or *Albizzia moluccana* has been cut down and the stumps left. *Ustulina zonata* grows on these dead stumps and, spreading along the lateral roots, comes in contact with the roots of tea bushes, which are thus infected. The fructifications of this fungus are frequently found in Ceylon on dead stumps of *Grevillea* as well as around the collar of tea bushes.

Although fructifications of *Ustulina zonata* have not yet been found on old stumps in Malaya, it is likely that the fungus often begins to grow on decayed stumps, from which it passes to the roots of rubber trees. On some of the older rubber estates on which the disease has been found, however, very few stumps remain, and it is possible that there are other modes of infection. The fact that the collar and tap root are sometimes the first parts of the tree to be affected points to the same conclusion. Several of the old trees seen to be affected by *Ustulina zonata* were previously attacked by white ants, and it is by no means impossible that the tw attacks are connected in some way, possibly by the ants bringing the fungus into the tree. With *Fomes semitostus* there are good grounds for thinking that white ants frequently follow an attack by the fungus, and it is possible that the reverse process occurs in the case of *Ustulina zonata*. We have, however, much to learn yet about the methods by which root parasites enter their hosts.

The disease of the collar and root system of rubber trees caused by *Ustulina zonata* is by no means easy to distinguish in the early stages, when there is some chance of dealing with it effectively, yet it is important that this should be attempted, as it is almost impossible to save the trees once the disease has made considerable progress. If the condition of the bark on one side of the collar of a tree arouses suspicion, it should be examined and, if found to be diseased, all discoloured tissues should be cut out and burnt and the exposed surfaces tarred; diseased lateral roots should be destroyed, unless they are large, when the unhealthy tissue should be excised. If the fungus has penetrated so far into the tree that it would fall if all the affected tissues were cut out, the tree is doomed, and efforts to save it will probably be useless; since, however, the fungus spreads but slowly, the tree may be kept in tapping until the end. Such trees should be cut out when they cease to yield latex in paying quantities. Although no external mycelium has yet been found around trees affected by this disease, it would be acting on the safe side to isolate an infected area by means of a trench. The fungus probably spreads from one rubber tree to another chiefly by contact of diseased roots against healthy ones.

With the drastic thinning out which has been recently carried out on many estates, and with the thinner planting of new areas than was formerly customary, it is important to remember that once the number of trees per acre falls below 80 to 100 the loss of a tree is a more serious matter than when an acre carried 150 to 200 trees. The smaller the number of trees remaining per acre the greater must be the attention paid in watching for the appearance of disease and treating it in the earliest stages, otherwise the yield from the estate will become seriously reduced. The disease caused by *Ustulina zonata* is a particularly insidious one, and rubber planters should be on their guard against it.

SUMMARY.

1. A disease of plantation rubber trees caused by *Ustulina zonata* and not hitherto recorded in Malaya is described. It is readily distinguishable from the root diseases caused by *Fomes semitostus*, *Sphærostilbe repens*, and *Hymenochæte noxia*.

2. The disease chiefly affects the collar and root system of old rubber trees, though a few five year old trees have been attacked by it.

3. The fructifications of the fungus are greyish brown or blackish plates, which are formed at the collar or on exposed lateral roots of diseased trees.

4. The fungus probably often begins to grow on decaying stumps, though in several trees it has been found to follow attacks of white ants.

5. It is important that the disease should be dealt with in the early stages by cutting out and destroying all discoloured tissues.

References.

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4.	Petch, T.	Root Diseases of Tea. Circ. Roy. Bot. Gard., Ceylon, Vol. 5, No. II., 1910.
5.	ditto	The Physiology and Diseases of *Hevea brasiliensis*. London, 1911, p. 159.
6.	ditto	The fungus diseases of *Hevea brasiliensis*. Rubber-recueil, International Rubber Congress, Batavia, 1914.
7.	Saccardo, P. A.	Sylloge Fungorum, I., p. 352.

Explanation of Figures.

Fig. 1. Photograph of a fructification of *Ustulina zonata* obtained from the base of a rubber tree.

Fig. 2. Photograph of the lower part of a diseased rubber tree bearing a fructification of *Ustulina zonata* (*f*). The tree has been opened up to shew the limits of diseased and healthy tissues (*d* and *h* respectively) above the collar. Latex (*l*) has exuded where healthy bark has been cut.

Fig. 3. Portion of rubber wood taken from the collar of an affected tree shewing black zones (natural size):—
- (*a*) long black zone near limit of diseased wood.
- (*b*) small black zones forming islands some distance from the chief black line.
- (*c*) diseased wood with brownish discolouration.

Fig. 4. Section of upper part of fructification, shewing conidial stage (material sent from Ceylon). × 630.
- (*a*) conidiophores.
- (*b*) portion of upper black layer.

Fig. 5. Mature ascospores of *Ustulina zonata*. × 330.

Fig. 6. Section of black zone in diseased wood of *Hevea brasiliensis* taken from the collar of an old tree. × 330.

Fig. 7. Section of black zone in wood of *Hevea brasiliensis* on which *Ustulina zonata* grew in pure culture. × 330.

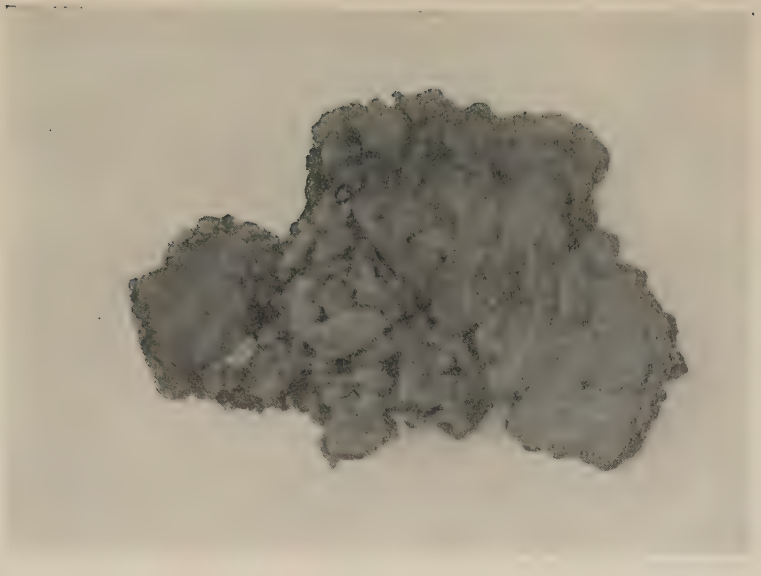

Fig. 1.

Fig. 2.

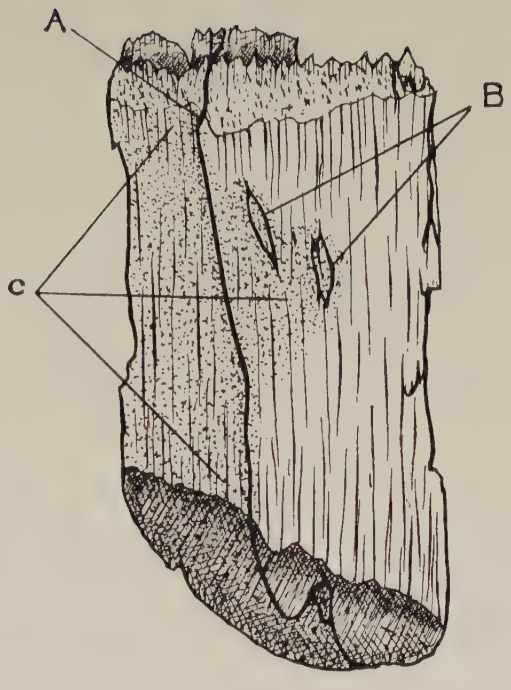

Fig. 3

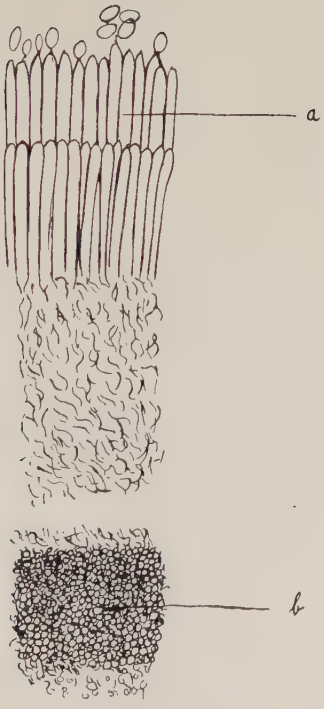

Fig. 4.

Fig 5.

Fig 6

Fig. 4